Claim 33 (withdrawn): A method for quantitatively assessing the extent of recrystallization occurring in frozen foods, and the impact of solution additives to inhibit or limit recrystallization according to the process as defined in claim 1.

Claim 34 (withdrawn): A method for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process as defined in claim 1.

REMARKS/ARGUMENTS

All claims pending in the application, namely 1-32 stand rejected under 35 U.S.C. §112, second paragraph as failing to

distinctly point out the invention; and under 35 U.S.C. §103(a) as being unpatentable over Olien (Ann. Rev. Plant Physiology, vol. 18, pages 337-408, 1967) in view of Warren et al. (U.S. Patent No. 5,118,792): Applicants respectfully disagree.

The Applicants have deleted in the original Abstract and provided a replacement one to overcome the Examiner's objections.

The Examiner has objected to the Applicant's inclusion of the hyperlink (www.ncbi.nlm.nih.gov) at page 145 of the specification. As described in the specification at page 145 lines 8 to 15, DNA sequence data was obtained from GenBank's database located on the National Center for Biotechnology Information's (NCBI) website. The NCBI is considered a national resource for molecular biology information and creates public databases, conducts research in computational biology, develops software tools for analyzing genome data and disseminates biomedical information. Applicants have clarified the specification to properly reflect this information. No new subject matter is being introduced by reference made to this site.

Claims 33 and 34 are withdrawn as being directed to a non-elected invention group.

No revision in inventorship is necessary in the current application.

Invention Summary

As described in the Background of the Invention section in the specification the presence of thermal hysteresis proteins (THP) is known to lower the non-equilibrium freezing point of water without lowering the melting point (equilibrium freezing point).

Page 3 starting at line 14 of the specification states:

If THP's are present, the temperature may be lowered as much as 5 to 6°C below the melting point (depending upon the specific activity and concentration of the proteins present) before noticeable crystal growth occurs. Thus, when THP's are added to a solution they produce a difference between the freezing and melting temperatures of the solution, and this difference has been termed "thermal hysteresis".

Further on page 3 starting at line 25:

This non-colligative freezing point depression means that antifreeze proteins are more efficient antifreezes on a molar basis, i.e. very low concentrations of AFP in pure solutions are known to have approximately five hundred times greater freezing point depression than colligative processes would predict. Given this, and their proteinaceous nature, they are an attractive alternative to the currently used de-icing solutions, since they are inherently environmentally friendly, non-toxic, biodegradable, and unlike the low molecular weight polyols, do not need to rely on high concentrations and colligative means to effect freezing point depression.

The thermal hysteretic behavior of antifreeze proteins is attributed to a specific protein-ice interaction that restricts ice growth, but not ice melt, hence creating a difference between the freezing and melting point of a solution. THP's are believed to create this thermal hysteretic effect via an adsorption-inhibition method. The protein absorbs (through hydrogen bonding and or hydrophobic interactions) to the surface of the ice crystal and slows or stops the growth of ice until the temperature is significantly lowered. Hence the freezing point of water is lowered by the binding action of the antifreeze protein. See specification at page 3 line 33 to page 4 line 13.

In general the present invention details recrystallization inhibition (RI) behavior of thermal hystersis proteins, in particular how extremely dilute solutions of THPS have been shown to inhibit the recrystallization of fine-grained ice samples in a concentration-dependent manner.

The high sensitivity of RI to the presence of THP's led Applicants to the present invention, as defined in the claims, which is a quantitative assay of THP activity using the recrystallization inhibition behavior. The extent of recrystallization in a fine-grained ice sample is quantified by estimating mean largest cross-sectional area for ice grains in the sample, thus providing the basis for a numerical assessment of RI. A number of different assay characteristics are addressed and detailed in the specification, including specificity of the RI assay with respect to THP's, ice grain size homogeneity within RI ice samples, RI assay sensitivities, applications of the assay, and assay automation.

As defined in currently amended claim 1, the invention particularly defines a recrystallization inhibition method for determining the presence, relative concentration, and or activity of thermal hysteresis proteins. A test solution made of a proteinaceous composition in a solvent is flash frozen; the temperature of the frozen solution is raised to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within the solution. The frozen solution is maintained at the annealing temperature for a length of time sufficient to allow for ice recrystallization. Changes in ice crystal grain size is monitored over time; and the presence of functional thermal hysteresis proteins in the solution is determined given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution.

Claim Rejections - 35 U.S.C. §112

Claims 1-32 have been rejected under 35 U.S.C. §112, second paragraph as failing to distinctly point out the subject matter of the invention.

Applicants have amended Claims 1, 3, 8, 9, 19 and 29 and address each of the Examiner's indefiniteness rejections as follows.

The Examiner has rejected Claim 1 as indefinite for the recitation of "relative concentration" stating the specification does not provide a standard for ascertaining the requisite concentration and therefore the claim should set forth the concentration amount. Applicants respectfully disagree.

The term "relative concentration" used in Claim 1 is well known to one of ordinary skill in the art and is not indefinite. In context of the present invention and as described in detail in the specification at page 80 to 113 and as illustrated in Figure 8 the "relative concentration" of a thermal hysteresis protein is the amount of protein needed for recrystallization inhibition behavior. Thus, the relative concentration would vary with the type of protein present in the proteinaceous composition.

The "/" in the term "and/or" has been deleted, so Claim 1 now reads "and or".

Claim 1 has also been amended to specifically define recrystallization as "ice" recrystallization.

The Examiner states that the last step in Claim 1 is a "mental interpretation and not a physical step *per se* and does not correlate ice crystal grain size with presence, relative concentration and/or activity". Applicants disagree.

As defined in the claims, the invention provides a "quantitative assay" using physical measurements and is not a "mental interpretation" as the Examiner suggests. Dependent claims 13, 14 and 16-25 clearly detail quantitatively how the presence of functional hysteresis proteins are determined in the invention method. Full support for this assertion is found in the specification at pages 8-113 and in Figure 8 et al.

Claim 1 is also rejected as "indefinite because [the claim] does not recite that the proteinaceous composition is [the] thermal hystersis [the] protein recited in the preamble and in view of claim 4." Claim 4 is rejected as lacking antecedent basis and for "requiring that the activity of the protein is known; and Claim 6 is rejected as providing a Markush Group inconsistent with the preamble of Claim 1. Applicants disagree and argue that these rejections are improper. These rejections are discussed together since Applicants believe the following argument presented should overcome them.

To clarify the invention provides a quantitative method for determining the presence, relative concentration and or activity of a thermal hysteresis protein which may be present in a proteinaceous composition. The presence of the thermal hysteresis protein is determined by the invention method and may have known activity as defined in Claims 4 and 5 or unknown activity as defined in Claim 7. See Applicants specification at page 13 lines 14-27. More specifically, Claim 6 defines the various proteinaceous compositions encompassed by the invention method. Therefore, Applicants believe that the Examiner's indefiniteness rejections of Claims 1, 4 and 6 are overcome and should be withdrawn.

Claim 2 is rejected as reciting the terms "or other isoomotic inorganic or organic solutions" as being open ended and undefined. Examples 1 to 5 described in the specification support inclusion of these terms.

Claim 3 has been amended to clarify that one control is "said solvent" as defined in Claim 1 and the other is a

control "solution" for non-specific recrystallization inhibition effects.

Claims 4 and 6 were rejected, but Applicants have addressed these rejections above.

Claim 7 is rejected as being indefinite for reciting "unknown functional antifreeze protein activity". Applicants disagree and again direct Examiner's attention to the specification at page 13 lines 20-27 which describes how the invention provides a method for determination of the presence of antifreeze proteins in unknown solutions or samples. The method determines the "presence, relative concentration and or activity" of a thermal hysteresis protein. The specification at pages 80 to 113, provide support for this assertion.

The rejections to Claims 8 and 9 have been overcome by amending the claims to clarify the proteinaceous composition and protein content respectively.

Claim 19 has been amended to correct the inconsistent bracket contained therein.

Claim 26 is rejected as being indefinite for reciting "known characterized parameters experimentally measured". Support for inclusion of these terms is found in the specification at pages 102 to 113.

Claim 29 has been amended to include the missing transitional phrase.

Claim 30 has been rejected because the term "high annealing temperature" is considered indefinite by the Examiner. Applicants direct Examiners attention to the specification at page 83, line 8 through page 84, line 25, which particularly describe the use of higher annealing temperatures. Therefore, Applicants believe that the recitation of "high annealing" temperatures is described in the specification and is not indefinite.

Applicants have addressed all the Examiner's indefiniteness rejections. In view of the amendments made and arguments presented Applicants believe all the Section 112 rejections have been overcome.

Claim Rejections - 35 U.S.C. §103(a)

Claims 1-32 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Olien (Ann. Rev. Plant Physiology, vol. 18, pages 337-408, 1967) in view of Warren et al. (U.S. Patent No. 5,118,792).

The Examiner acknowledges that Olien does not explicitly teach a method for determining the presence of the hysteresis protein, but she argues that "the activity is monitored". She further cites a secondary reference to Warren as disclosing a method for screening antifreeze polypeptides by monitoring inhibition of ice crystal growth via refreezing on a cooled metal block (the splat assay). The Examiner's position is that the combination of these teachings would make the present invention obvious to one of ordinary skill in the art at the time of the invention. Applicants respectfully disagree.

The Examiner describes Olien as disclosing a method to examine antifreeze properties of plant extracts, through monitoring the thermodynamics and kinetics of ice crystal growth in a film during a "refreezing" process (Olien page 396). The method involves an initial freezing, followed by thawing to about three fourths (partial thaw, so that some ice remains at the start of the refreezing bout to nucleate and avoid supercooling), and then the sample is refrozen slowly, and the subsequent refreezing of the remaining liquid water is then monitored and kinetics of heat removal evaluated over a series of re-freezing tests with different freezing rates during the refreezing process.

The Examiner argues that "this method is a measure of recrystallization inhibition". Applicants respectfully submit that this assertion is incorrect. This method is neither an assessment of recrystallization inhibition, or of the process of recrystallization itself. Rather, as Olien states, it is an assessment of an equilibrium freezing process (not a thawing process as would be the case for recrystallization).

However, Applicant's acknowledge that the Examiner is correct in her statement that Olien provides "a form of monitoring thermal hysteresis". As discussed earlier under the Invention Summary, thermal hysteresis is the difference between the equilibrium melting and freezing points of a solution. By definition, the equilibrium melting and freezing points are identical. Yet, in solutions containing antifreeze proteins, the non-equilibrium freezing point is lowered without lowering the melting point, creating a "thermal hysteresis (TH)".

Albeit, for Olien in 1967, this term, and the significance of non-equilibrium freezing point depressing activity of proteinaceous antifreezes was yet to be discovered, given that it wasn't until 1969 that DeVries and Wohlschag, first reported the thermal hysteresis phenomenon of a specific antifreeze protein from a marine teleost fish (Science 163: 1073-1075 (1969)). And specifically for winter cereals (as is the case with Olien), it wasn't until 1992, that Griffen et al (Plant Physiology vol. 100, pgs 593-596 (1992)) identified the presence of proteainaceous antifreezes in winter rye, most likely the contributing source to the non-equilibrium freezing instances originally observed in Olien. Nevertheless, the basic procedure that Olien presents (i.e. monitoring ice crystal growth in a film during a "refreezing" process, involving first a partial thaw, and then a bout of refreezing) is the basic concept behind two, current day methods for assessing thermal hysteresis; the nanoliter osmometer, requiring microscopic observations (Chakrabrartty and Hew, 1989 J. Biological Chemistry, Volume 264, pgs 11313-11316), and the rather sophisticated and costly, thermodynamic assessments involving differential scanning calorimetry (DSC) (Hansen and Baust, Biochimica et Biophysica Acta. Vol. 957, pgs. 217-221, 1988). Again, as with Olien, these current methods are evaluating an equilibrium freezing process, and not the phenomena of recrystallization, or its inhibition.

Unlike the present invention, the methods detailed in Olien focus on the assessment of freezing processes, and not recrystallization, a thawing process (check best ref, e.g. *Mazur, American Journal of Physiology vol. 247, pg C125-C142, 1982*).

The Examiner states the secondary reference to Warren et al discloses screening the antifreeze polypeptides by monitoring inhibition of ice crystal growth via refreezing on a cooled metal block, the splat assay (column 4, lines 48-62 and example 3). As will be clear from the discussion that follows, the splat assay does not assess "refreezing" as does the present invention.

Warren's use of the splat assay is to screen for antifreeze polypeptides (AFPs) via assessing the extent of recrystallization occurring in a frozen sample, and any inhibition of recrystallization (RI) presumably attributed to the presence of AFPs, is the basic premise behind the need for the present invention.

Applicants findings show that the splat assay, as detailed in Warren et al is seriously flawed with respect to

1) antifreeze protein specificity (i.e. there are numerous circumstances under which the splat assay gives a false positive indication for the presence of AFPs, when in fact, no AFPs are present. This, of course, is extremely problematic.), 2) it is totally subjective in its visual monitoring of ice crystal size changes, offering no true quantitation or standardization, and 3) it lacks any means of referencing the degree of recrystallization inhibition observed, to that of specific AFP concentrations in unknown samples. Each of these problems demands solutions.

It is well settled that the mere fact that the prior art could be modified to form the invention would not make the modification obvious *unless the prior art suggested the desirability of the modification. In re Laskowski,* 10 USPQ2d 1397, 1398 (Fed. Cir. 1989); *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984).

The primary reference to Olien does not teach or suggest the recrystallization inhibition method of the invention which provides a quantitative method for determining the presence, relative concentration and or activity of thermal hysteresis proteins. In fact, as stated earlier, it teaches away from the present invention in disclosing an equilibrium freezing process (not a thawing process as would be the case for recrystallization).

Therefore, there would be no motivation to combine the teaching of Olien with the secondary reference to Warren et al. to obtain the method of the present invention.

Even it this were so, the present invention does not disclose the "splat assay" per se as described in Warren et al, rather, the present invention provides substantial and significant improvements in the use of the splat assay approach to correct each of these three flaws discussed earlier herein. The invention also details further recrystallization inhibition (RI) detection spin off approaches that add more rapid RI screening venues and means for upscaling to multiple sample testing, while still ensuring antifreeze protein specificity, truly quantifiable and non-biased RI activity assessments and standardizations, and corresponding detection of specific AFP concentrations.

Applicants contend that the Examiner has not made a prima facie case of obviousness and that the rejections of Claims 1-32 should be withdrawn.

In view of the above arguments, the Applicants believe they have overcome both the indefiniteness and obviousness rejections. No new matter has been introduced by this Amendment. Applicants submit that this application is now in condition for allowance. Applicants hereby request reconsideration of this application and allowance of pending claims 1-32. If a telephone interview would be useful to advance this case, then the Examiner is invited to telephone the undersigned.

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On 6/8/04
(Date of Deposit)

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